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by

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16. ABSTRACT

Enlarged kidneys and hydronephroses were observed in day 15 postpartum (pp) CD-1 mouse pups from dams treated with 10 or 50 mg HCB/kg body weight on days 6-16 of gestation. Additional studies showed that enlarged kidneys occurred as well on days 1 and 20 pp. CD rat pups from dams exposed to 10 mg HCB/kg body weight on days 15-20 of gestation had enlarged kidneys on postpartum day 1 but not on days 10 or 20. In the CD rat, there was a significant increase in the kidney-to-body-weight ratio and the liver-to-body-weight ratio for the HCB exposed pups at all three time periods. Pre- and postnatal exposure to HCB resulted in renal maldevelopment in CD-1 mice and CD rat in terms of enlarged kidneys and hydronephrosis.

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SUMMARY

Enlarged kidneys and hydronephroses were observed in day 15 postpartum (pp) CD-1 mouse pups from dams treated with 10 or 50 mg HCB/kg body weight on days 6-16 of gestation. Additional studies showed that enlarged kidneys occurred as well on days 1 and 20 pp. CD rat pups from dams exposed to 10 mg HCB/kg body weight on days 15-20 of gestation had enlarged kidneys on postpartum day 5 but not on days 10 or 20. In the CD rat, there was a significant increase in the kidney-to-body-weight ratio and the liver-to-body-weight ratio for the HCB exposed pups at all three time periods. Pre- and postnatal exposure to HCB resulted in renal maldevelopment in CD-1 mice and CD rat in terms of enlarged kidneys and hydronephrosis.

INTRODUCTION

Hexachlorobenzene (HCB) is a global pollutant which, due to its persistance, has been detected in human milk, blood and adipose tissue as well as in plants and many species of wildlife, including mammals, birds and fish. HCB has been shown to cross the placenta and accumulate in the fetus in a dose-dependent manner (Villeneuve and Hierlihy, 1975; Courtney and Andrews, 1985). Other studies have shown that HCB can cross readily in the milk during lactation and accumulate in the neonate while depleting the concentrations in the mother (Grant et al., 1977; Courtney and Andrews, 1985). Khera (1974) varied the days of exposure to pregnant Wistar rats from early to late and throughout gestation. He found that HCB exposure resulted in an increased dose related incidence of extra ribs and sternal defects and was therefore teratogenic in the Wistar rat. Mendoza et al., (1975) treated pregnant

Wistar rats with HCB and found that on day 18 postpartum, neonates had a significantly increased liver weight but a decreased kidney, brain, spleen and heart weight. Grant et al., (1977) performed reproductive and viability studies with CD rats and found that HCB at higher concentrations significantly reduced fertility, viability till five days of age, and viability from five to twenty one days of age. HCB produced stillbirths and a high level of postnatal mortality. Villeneuve et al., (1974) performed teratology studies on New Zealand rabbits by treating pregnant rabbits throughout gestation with HCB. He found no malformations or fetotoxic effects in this species. Courtney et al., (1976) exposed pregnant CD-1 mice to HCB during gestation and upon examining the fetuses on day 18, found an increase in the incidence of enlarged renal pelves, cleft palate and renal agenesis. Teratology studies with C57B1/6 mice and CD rats were performed using pure PCNB or technical PCNB containing 10% HCB. No teratogenic response was found with the pure PCNB, but the contaminated PCNB exposure resulted in a high incidence of renal agenesis in the mice but no effects in the CD rats. In order to determine the importance of these kidney changes on the ability of the pups to survive after birth, kidneys were weighed and examined during postnatal studies in both CD-1 mice and CD rats.

METHODS

Pregnant CD-1 mice and CD rats with known insemination dates were purchased from Charles River Laboratories, Inc., Wilmington, MA. Detection of a vaginal plug or sperm denoted day 0 of gestation. During gestation, rats were housed 3 per cage and mice were housed 5 per cage in polycarbonate cages containing heat-treated pine shavings

(Betta Chips, North Eastern Products, Inc., Warrenburg, NY) and given feed (Wayne Lab Blox, Allied Mills, Inc., Chicago, IL) and filtered water (automatic watering system) ad libitum. An ambient temperature of 22 ± 2°C, a relative humidity of 55 ± 5%, and a 12 hour light-dark cycle were provided. Cages were housed under spun polyester filter bonnets. A sentinel mouse program monitored the animal holding rooms for the presence of rodent viruses, bacteria and other pathogens. On the day before parturition, the mice and rats were housed one per cage and were allowed to deliver and nurse their young.

HCB was obtained from the British Drug Co., Poole, Eng. at a purity of > 99%. HCB was administered intragastrically as a solution in corn oil using volumes of 0.4 ml per rat and 0.2 ml per mouse. At the highest concentration in the rat, the solution was warmed to assure solubilization of HCB. In three postnatal CD-1 mc se studies and two postnatal CD rat studies with HCB, the kidneys were examined for renal development. In the first study, the mice were treated with 0, 0.1 or 10 mg HCB/kg body weignt on days 11-17 of gestation and whole litters examined on day 1 or 6 postpartum. In the second study, mice were treated with 0, 10 or 50 mg HCB/kg body weight on days 11-16 of gestation. Pups from each litter were randomly selected for examination on days 5, 10 or 20 pp. in the third mouse study, mice were treated with 0, 10 or 50 mg HCB/kg body weight on days 6-16 of gestation and the neonates examined on day 15 pp. In the first rat study, the dams were treated on days 15-20 of gestation with 0 or 10 mg HCB/kg body weight and neonates randomly selected for examination on days 5 or 10 pp. In the second rat study, dams were treated on days 10-20 of

gestation with 0 or 10 mg HCB/kg of body weight and whole litters examined on day 20 pp.

Although the dams were exposed during gestation, it can be considered that the pups were exposed to HCB both pre- and postnatally (Fig. 1). Figure 1 is a composit of a number of studies in our laboratory (Courtney, K.D., et al., 1979; Courtney, K.D. and Andrews, J.E., 1985). Bar A indicates the amount of HCB which was found in the gestational day 17 fetuses which were from mothers treated with HCB during gestation. Bar B represents the HCB found in neonates which were cross-fostered to dams which had not been treated with HCB. The neonates nursed until day 4 pp and their HCB content was not changed. Bar C represents neonates from control dams which were cross-fostered on day 3 pp to dams exposed to HCB during gestation. In these neonates, a larger amount of HCB was transferred to the pups than to those exposed during gestation, i.e. bar A. Bar D represents pups from HCB treated dams which were left with their dams through day 4 of lactation. A much greater amount of HCB was found in these pups than was found in those exposed only during gestation or only during lactation.

All values presented are the means and standard errors (SE) using the litter as the unit of analysis. Statistical analyses were performed using ANOVA and student's t test.

RESULTS

Results from exposure of CD-1 mice on gestational days 11-17 with 0, 1.0 or 10.0 mg HCB/kg body weight are presented in Table I. All pups from a litter were examined on day 1 or 6 pp. The kidney

weights in this table are for the individual kidney. There was no apparent HCB toxicity in the dams since there were no significant differences in the body, liver or kidney weights. In contrast, at the highest dose level of HCB, there was a significant increase in body weights of the pups on both days 1 and 6 pp. There was also a significant increase in the kidney weights of these pups. In the day 1 pups at the highest dose level, there was a significant increase in the kidney to body weight ratio but by day 6, there was a significant decrease in the ratios at both dose levels. In the 2nd study (Fig. 2), dams were treated on days 11-16 of gestation with HCB and three pups from each litter randomly selected for examination on days 5 and 10 pp. The remaining pups in the litter were examined on day 20 pp. There were no significant differences in the body weights, individual kidney weights or kidney to body weight ratios on days 5 or 10 pp. By day 20 though, there was a significant increase in body weight and kidney weight of the high dose group compared to the controls. There was no increase in the kidney-to-body-weight ratio in this high dose group, but there was a significant increase in the kidney to body weight ratio at the 10 mg HCB level.

In the next study, CD-1 dams were exposed to 0, 10 or 50 mg HCB/kg body weight on days 6-16 of gestation and their pups examined on day 15 pp (Table 2). There were no significant differences in the body weights of the exposed compared to the control pups. The combined kidney weights, and kidney to body weight ratios were elevated significantly at the 50 mg dose level. The ranges for the combined kidney weights of the treated pups were far greater than those observed for the controls. The kidney data were examined further by

evaluating kidneys weighing greater than 100 mg and those weighing less than 100 mg as shown in Table 2. There was only one pup from one litter of the control pups which had kidneys weighing greater than 100 mg. Many kidneys from the HCB exposed pups weighed greater than 100 mg. Three hydronephrotic kidneys were found in the 10 mg HCB treatment group. Figure 3 shows a hydronephrotic kidney on the right and a control normal kidney on the left. Although there were many kidneys weighing more than 100 mg, in the HCB exposed groups, most kidneys weighed less than 100 mg. The weights of these kidneys from HCB exposed pups were significantly greater in a dose related manner than the weights for kidneys from the control pups.

CD rat dams were exposed to 0 or 10 mg HCB/kg body weight on days 15-20 of gestation and 5 neonates randomly selected and examined on day 5 pp. The remainder of the pups were examined on day 10 pp (Table 3, Study 1). There were significant increases in both liver and kidney weights and liver- and kidney-to-body-weight ratios on day 5 pp. By day 10 though, there were no differences between the treated and control kidney weights, although the liver weight of the HCB treated group was still significantly elevated which was reflected in a significantly increased liver-to-body-weight ratio. In Rat Study II (Table 3), dams were exposed to HCB on the last seven days of gestation and the whole litter examined on day 20 pp. There were no significant differences in the kidney weights of the HCB exposed pups compared to the control pups. However, two pups from the HCB exposed group were observed to have hydronephrotic kidneys. Pup body weights were not significantly different yet the kidney-to-body-weight ratios were significantly elevated in the HCB treatment group. The

liver weights were not significantly different from the controls yet the liver-to-body-weight ratios were significantly elevated in the HCB group.

DISCUSSION

Pre- and postnatal exposure to HCB caused enlarged kidneys in the CD-1 mouse pups. Enlarged kidneys were seen on days 1, 15 and 20 pp. It is not known if the effect on day 1 pp is the same as the effect seen on days 15 and 20. During late gestational development, the kidney goes through a stage in which the pelvis appears large. This lasts for approximately 24-48 hours and then the size of the pelvic area diminishes. In delayed development, the condition of enlarged renal pelvis may be prolonged throughout the first few postnatal days. It is not known if the condition of the enlarged renal pelves will become permanent, progress to hydronephrosis or revert to the normal condition. This ambiguity makes it difficult to assess with assurity enlarged kidneys from day 1 to day 6 pups, especially when the body weights of the pups have increased.

Often a significant observation can be obscured by the variability of some of the data as seen in Table 2. Although the kidney weights were significantly elevated at the highest dose level, the mean weight for the kidneys at the lower dose level was not significantly different from the controls due to high variability. When the kidneys weighing greater than 100 mg were removed from the table, a dose related increase in kidney size was revealed. Only one kidney from 84 control neonates was found to weigh greater than 100 mg whereas 26 of 114 neonates at the lower dose level and 13 of 39 at the higher dose level

exceeded this value. Other than the 3 frank hydronephrotic kidneys found in 3 pups, many of the enlarged kidneys appeared normal and the cause of the renal enlargement was not known.

An increase in kidney weight was noted in day 5 CD rat pups but on days 10 and 20 the kidney weights were not significantly different from control values. The 2 hydronephrotic kidneys observed cannot be ascribed to HCB exposure without additional studies.

The observation of significant increases in liver-to-body-weight ratio as seen in the neonatal rat (Table 3) is consistent with observations of other investigators (Grant et al., 1977; Mendoza et al., 1975) and is a condition often seen after exposure to chlorinated aromatic hydrocarbons. The compounds such as HCB, PCB and dioxins have all been shown to induce or increase the microsomal proteins of the endoplasmic reticulum and result in increased microsomal enzyme activities (HCB: Mendoza et al., 1977; Carleson and Tardiff, 1976; Turner and Green, 1974; PCB: Chu et al., 1977; Ando et al., 1984; Parkinson et al., 1980; TCDD: Poland and Knutson, 1982).

In addition, the pattern of teratogenicity observed for HCB, cleft palate and renal anomalies (Courtney et al., 1976), is similar to the pattern observed in TCDD teratogenicity, cleft palate and renal anomalies (Courtney and Moore, 1971; Moore et al., 1973). TCDD exposure (pre- and/or postnatal) has been shown to result in an increased incidence of postnatal hydronephrosis (Moore et al., 1973). This report was only concerned with prenatal treatment of the dams and the subsequent effect on the pups. It is not known if the mechanism of production of the hydronephrotic kidneys from HCB and TCDD exposure is similar although the pattern of prenatal and postnatal effects are similar.

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TABLE 1

POSTNATAL EFFECTS OF HCB ON CD-1 MOUSE DAMS AND NEONATES FOLLOWING HCB EXPOSURE ON DAYS 11-17 OF GESTATION.

HCB mg/kg	No. Neonates (No. Litters)	ADULT			NEONATE		
		Body Wt (g)	Liver Wt. (g)	Kidney Wt. (g)	Body Wt. (g)	Kidney Wt. (mg)	Kidney/ Body Wt X 100
				DAY 1 POSTPARTUM			
0	78 (9)	30.7 ± 1.0[b]	1.87 ± 0.07	0.34 ± 0.03	1.59 ± 0.07	7.01 ± 0.50	0.43 ± 0.02
1.0	88 (11)	28.4 ± 0.3	1.60 ± 0.03	0.29 ± 0.01	1.59 ± 0.04	7.45 ± 0.30	0.47 ± 0.01
10.0	34 (5)	31.1 ± 0.7	1.76 ± 0.10	0.37 ± 0.02	1.91 ± 0.05[a]	10.00 ± 1.14[a]	0.53 ± 0.04[a]
				DAY 6 POSTPARTUM			
0	34 (6)	33.1 ± 0.9	2.46 ± 0.15	0.40 ± 0.02	3.39 ± 0.43	21.3 ± 1.2	0.67 ± 0.05
1.0	46 (5)	38.6 ± 2.2	2.72 ± 0.13	0.46 ± 0.02	4.02 ± 0.16	21.0 ± 1.2	0.52 ± 0.01[a]
10.0	57 (7)	35.5 ± 2.1	2.66 ± 0.18	0.43 ± 0.03	4.46 ± 0.27[a]	25.6 ± 1.7[a]	0.57 ± 0.02[a]

[a]Significantly different from control, $p < 0.05$

[b]Values are means ± SE

TABLE 2

POSTNATAL EFFECTS OF HCB ON CD-1 MOUSE PUPS ON DAY 15 PP FOLLOWING HCB EXPOSURE ON DAYS 6-16 OF GESTATION.

HCB mg/kg	No. Pups (No. Litters)	Pups Wt (g)	Kidney Wt. (mg)	Kidney/ Body Wt. (%)	Kidney Wt. Range
		ALL DATA			
0	84 (7)	2.19 ± 0.06[b]	78.0 ± 2.3	3.58 ± 0.18	46-120
10	114 (11)	2.24 ± 0.06	100.6 ± 9.9	4.25 ± 0.19	53-506
50	39 (5)	2.23 ± 0.04	100.0 ± 6.3[a]	4.48 ± 0.24[a]	51-141
		DATA FOR KIDNEYS > 100 mg			
0	1 (1)	2.39	120	5.02	120
10	26 (6)	2.40 ± 0.10	124 ± 13	5.23 ± 0.40	101-506
50	13 (4)	2.20 ± 0.05	110 ± 4	4.95 ± 0.15	103-141
		DATA FOR KIDNEYS < 100 mg			
0	83 (7)	2.19 ± 0.06	78.0 ± 2.3	3.58 ± 0.18	46-100
10	88 (9)	2.17 ± 0.04	85.0 ± 1.7[a]	3.91 ± 0.05	53-100
50	26 (4)	2.25 ± 0.07	90.3 ± 2.5[a]	4.00 ± 0.10	51-100

[a]Significantly different from control, $p < 0.05$

[b]Values are means ± SE

TABLE 3

POSTNATAL EFFECTS OF HCB ON CD-1 RAT PUPS
FOLLOWING HCB EXPOSURE ON DAYS 15-20 OF GESTATION.

HCB mg/kg	No. Pups (No. Litters)	Pup Wt (g)	Liver Wt (mg)	Liver/ Body Wt. (%)	Kidney Wt. (mg)	Kidney/ Body Wt. (%)
RAT STUDY I						
			DAY 5 POSTPARTUM			
0	15 (3)	11.7 ± 0.1[b]	319 ± 12	2.8 ± 0.1	46.0 ± 3.9	0.39 ± 0.03
10	30 (6)	12.4 ± 0.5	425 ± 20[a]	3.4 ± 0.1[a]	87.8 ± 4.3[a]	0.71 ± 0.01[a]
			DAY 10 POSTPARTUM			
0	21 (3)	25.2 ± 0.3	722 ± 40	2.8 ± 0.2	188.3 ± 8.9	0.75 ± 0.04
10	41 (6)	27.3 ± 1.1	875 ± 40[a]	3.2 ± 0.0[a]	172.0 ± 7.3	0.63 ± 0.01[a]
RAT STUDY II						
			DAY 20 POSTPARTUM			
0	100 (8)	33.0 ± 2.8	1080 ± 78	3.3 ± 0.1	210.0 ± 15	1.29 ± 0.04
10	109 (9)	30.6 ± 0.9	1160 ± 35	3.8 ± 0.1[a]	212.0 ± 5.7	1.40 ± 0.03[a]

[a]Significantly different from control, $p < 0.05$

[b]Values are means ± SE

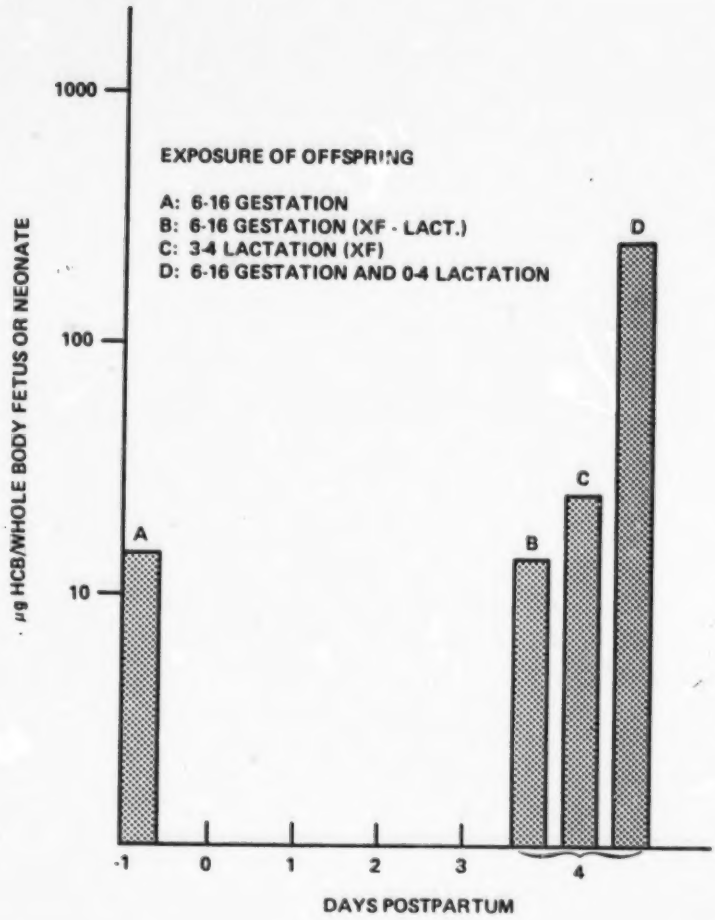

Figure 1. Microgram HCB per CD-1 mouse fetus or neonate with various exposures to dams which had been treated IG with 50 mg HCB/kg body weight on days 6-16 of gestation. (XF is cross fostered).

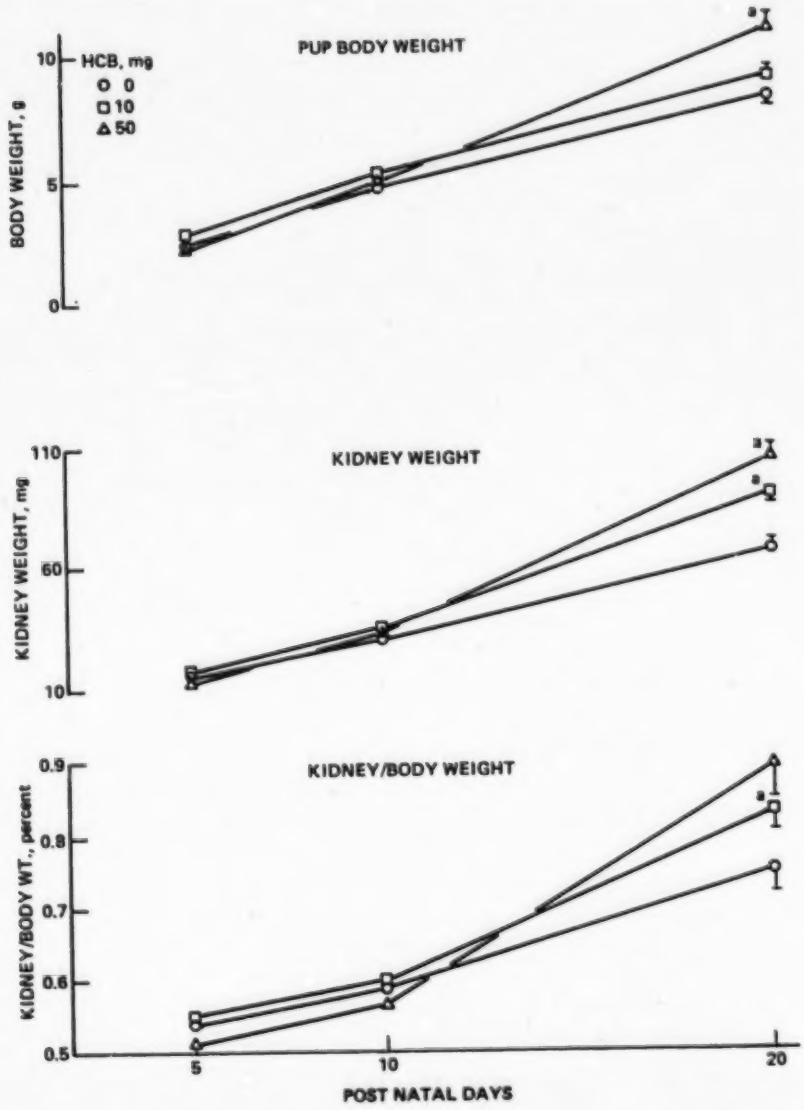

Figure 2. Postnatal kidney and pup weights and kidney-to-body-weight ratio of CD-1 mouse pups from dams treated with 0, 10 or 50 mg HCB/kg body weight on days 11-16 of gestation. Values are means ± standard errors for 3-8 litters per time period. "a" denotes a statistically significant difference from the control values at $p < 0.05$.

Figure 3. Hydronephrotic kidney (right) from CD-1 mouse pup exposed to dam treated with 10 mg HCB/kg body weight on days 11-17 of gestation. Control kidney is on the left.